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Simultaneous determination of pseudoephdrine, pheniramine, guaifenisin, pyrilamine, chlorpheniramine and dextromethorphan in cough and cold medicines by high performance liquid chromatography

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ABSTRACT

A new simple, rapid and sensitive liquid chromatographic method has been developed and validated for the simultaneous determination of pseudoephdrine, pheniramine, guaifenisin, pyrilamine, chlorpheniramine and dextromethorphan in cough and cold pharmaceuticals. The separation of these compounds was achieved within 13 min on a Kromasil C18 column using an isocratic mobile phase consisting of methanol–dihydrogenphosphate buffer at pH 3 (45:55, v/v). The analysis was performed at a flow rate of 1 mL min^{-1} and at a detection wavelength of 220 nm. The selectivity, linearity of calibration, accuracy, within and between-days precision and recovery were examined as parts of the method validation. The concentration–response relationship was linear over a concentration range of 5–50 μg mL^{-1} for pseudoephdrine, pheniramine, chlorpheniramine and 50–600 μg mL^{-1} for guaifenisin, pyrilamine, dextromethorphan, methylparaben and sodium benzoate with correlation coefficients better than 0.998. The standard deviations of the intraday and interday were all less than 2%. The proposed liquid chromatographic method was successfully applied for the routine analysis of these compounds in different cough and cold pharmaceutical preparations such as syrups, capsules, tablets and sachets. The presence of preservatives (sodium benzoate and methylparaben) and other excipients did not show any significant interference on the determination of these compounds.

1. Introduction

Cough and cold pharmaceutical preparations are one of the most extended formulations in the world and have got many pharmaceutical forms: syrup, suspension, sachets, capsules and tablets [1]. These preparations represent complex formulations containing several active ingredients and a broad spectrum of excipients such as flavoring agents, saccharose or aspartame, acidulants, natural or artificial coloring and flavoring agents, dyes, sweeteners and preservatives [2,3]. The majority of these ingredients are present as a mixture of basic nitrogenous amino compounds and their separation in pharmaceutical forms is quite complicated due to similarities of their physical and chemical properties [4]. The combination of antihistamine such as pyrilamine maleate (PA) and chlorpheniramine maleate (CLP) is used to overcome the allergic effects and reduce or relieve cold symptoms [5]. Pheniramine maleate (PHEN) and pseudoephedrine hydrochloride (PE) are widely used in combination with other drugs for the clinical treatment of common cold, sinusitis, bronchitis and respiratory allergies [6]. Dextromethorphan hydrobromide (DEX) and guaifenisin (GUA) were used as cough suppressants antitussive for the relief of non-productive cough and cold preparations [7]. The most common formulation can be either liquid or suspension that requires the addition of preservatives such as sodium benzoate (SB) or methylparaben (MP). All these components have different polarities and exist in very different proportion. Due to these characteristics and because of diverse properties inherent to their formulation, these preparations offer an analytical problem [8].

Several methods are reported in the literature for the determination of these compounds in pharmaceuticals and in physiological fluids [9–18]. High performance liquid chromatography (HPLC) with UV or fluorescence or mass detection, is the most used techniques [1–3,5,6,9,11,14,15,17–24]. Other techniques including ultraviolet–visible (UV–vis) spectroscopy, thin layer chromatography, gas chromatography, GC/MS, capillary electrophoresis and multivariate spectrophotometric method [4,23–27], have been used to determine few of these compounds. However, to the best of our knowledge, no analytical methods have been reported for the simultaneous determination of all compounds in the presence of

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preservatives and excipients, which might interfere with the analysis of the active ingredients.

In addition, the majority of the published HPLC methods use an ion pairing agent, in order to reduce peak tailing and enhance separation of basic drugs on silica-based columns. These chromatographic systems are highly suitable but require the use of hydrophobic additives either cationic, such as trimethylamine, hexylamine or anionic, such as the alkyl sulfonate. However, these additives are costly and tend to adsorb very strongly on the stationary phase, leading to difficulty in recovering initial column properties. Furthermore, the use of ion pairing agents in the mobile phase will enhance the retention time of most compounds and as a consequence an increase in the analysis time will be observed.

The aim of this study was to develop a new, rapid, accurate and selective isocratic HPLC method for the simultaneous determination of six of the most commonly used active ingredients found in cough and cold medicines (PE, PHEN, GUA, PA, CLP and DEX) as well as the preservatives (SB and MP) in the presence of other recipients without using ion pairing additives.

2. Experimental

2.1. Chromatographic conditions

A HP Agilent liquid chromatograph Model 1200, equipped with a quaternary pump, an auto-sampler, a vacuum degasser, a diode-array UV-detector, a column thermostat and a data station (HP Chemstion) was used. The detection of analytes was monitored at 220 nm by a diode-array UV-detector. The separation was achieved on a Kromasil LC18 column (150 mm × 4.6 mm I.D., 5 μm particle size) using a mobile phase containing 45:55 (v/v) methanol/0.1 mol L^{-1} KH_2PO_4 buffer. The analysis was performed at a flow rate of 1 mL min^{-1}. The pH of the buffer was adjusted with orthophosphoric acid or sodium hydroxide. Prior to any analysis, the mobile phase was degassed and filtered using 0.45 μm filters. The system was equilibrated with the mobile phase before injection.

2.2. Reagents and chemicals

Working reference standard of guaifenisin (GUA), chlorpheniramine maleate (CLP), pheniramine maleate (PHEN), pseudoephdrine hydrochloride (PE), dextromethorphan hydrobromide (DEX), pyrilamine maleate (PA), sodium benzoate (SB) and methylparaben (MP) were purchased from Sigma–Aldrich. HPLC grade methanol (MeOH) was obtained from Prolabo (Paris, France). Ultrapure water was drawn from a Diamond Reverse Osmosis System (United Kingdom). Analytical grade potassium dihydrogenphosphate, orthophosphoric acid and sodium hydroxide were obtained from Prolabo (Paris, France).

The commercialized pharmaceutical products used are detailed below:

Nortussine syrup for children (400 mg GUA, 100 mg DEX, 100 mg PA and 50 mg MP for 100 mL) and *nortussine syrup for adult* (200 mg DEX, 200 mg PA and 200 mg SB for 100 mL) were manufactured by Pharmagreb, Tunisia.
Tussipax syrup cough suppressant for children (400 mg GUA, 100 mg DEX and 150 mg MP for 100 mL) was manufactured by Opalia, Tunisia.
Rhinostop syrup (1 mg CLP, 20 mg PE, and 100 mg paracetamol for 100 mL) was manufactured by Simed.
Sudafed syrup (600 mg PE for 100 mL) was manufactured by LABORATOIRE GlaxoSmithKline, France.

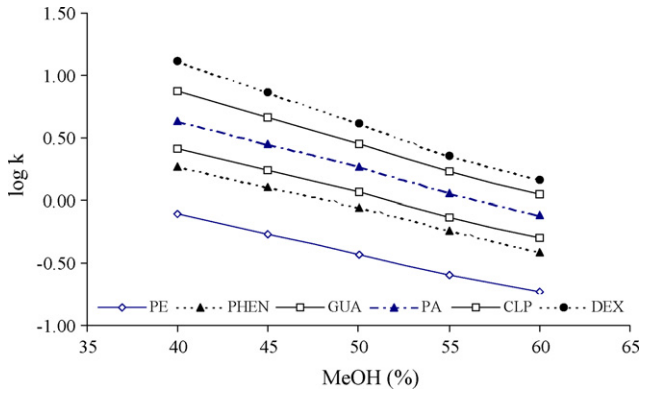

Fig. 1. Effect of methanol concentration on the retention of pseudoephdrine (PE), pheniramine (PHEN), guaifenisin (GUA), pyrilamine (PA), chlorpheniramine (CLP), and dextromethorphan (DEX). Mobile phase: methanol:phosphate buffer (0.1 mol L^{-1}); column: Kromasil C18; detector = 220 nm; flow rate: 1 mL min^{-1}.

Fervex granuled powder for oral solution (25 mg CLP, 500 mg pracetamol, and 200 mg ascorbic acid per sachet) was manufactured by Bristol–Myers–Squibb, UPSA, Tunisia.
Gripex powder for oral solution (25 mg CLP, 500 mg pracetamol, and 200 mg ascorbic acid per sachet) was manufactured by Galpharma, Tunisia.
Rhinofebral capsule (3.2 mg CLP, 240 mg acetaminophen, and 100 mg ascorbic acid per tablet) was manufactured by Crepharm Bessay McNeil SAS, France.
Rhinostop tablet (2.5 mg CLP, 60 mg PE, and 250 mg paracetamol for one tablet) was manufactured by SAIPH, Tunisia. All these medicines were kindly provided by local manufactured or purchased at local drug stores.

2.3. Preparation of solutions

Stock standard solutions of PE, PHEN, CLP (200 μg mL^{-1} each) and of GUA, PA, DEX, SB, MP (1000 μg mL^{-1} each) were prepared in ultrapure water. Working standard solutions (5–50 μg mL^{-1} for PE, PHEN, CLP and 50–600 μg mL^{-1} GUA, PA, DEX, SB and MP) were freshly prepared by serial dilutions of the stock standard solutions.

Commercialized pharmaceutical samples were prepared as follows:

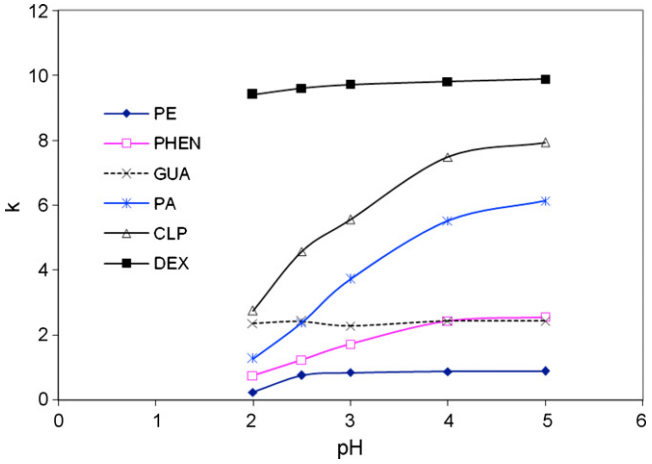

Fig. 2. Variation of the retention of pseudoephdrine (PE), pheniramine (PHEN), guaifenisin (GUA), pyrilamine (PA), chlorpheniramine (CLP), and dextromethorphan (DEX) with the pH of the mobile phase. Mobile phase: 45:55 (v/v) methanol:phosphate buffer (0.1 mol L^{-1}); column: Kromasil C18; detector = 220 nm; flow rate: 1 mL min^{-1}.